Detection of Cyclin D1 in Formalin-Fixed, Paraffin-Embedded, Rat Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
DAB Chromagen
Hematoxylin

Antibody Information:

Blocking Serum: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog# 008-000-001

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog# SP-2001

Primary Antibody Mouse anti-Cyclin D1
Dako Corporation
Carpinteria, CA 93013
www.dakousa.com
1-800-235-5763
Catalog# M7155

Negative control serum: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog# 015-000-001

Secondary antibody: Biotinylated Horse anti-mouse IgG
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # BA-2001

Label antibody: Peroxidase –conjugated Streptavidin SS Label
Biogenex
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Rat GI tract
Stain Localization: Nuclear. According to the Dako specification sheet, cytoplasmic staining does occur. If no nuclear staining is present with the cytoplasmic staining, score stain as non-specific.

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking Techniques using the decloaker.
   Add 500 ml of distilled water to the pan of the decloaker.
   Place full rack of slides in 200 ml of EDTA (1:5) and place in the decloaker.
   Decloak for 5 minutes. Pressure_____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. Temperature before cooling _____
   Rinse in distilled water two times for 3 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Block with 10% Normal Horse Serum block and incubate for 20 minutes at room temperature.
   Lot#_____ Reconstituted Date_____

   DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block
   Lot#_____ Exp Date_____ New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X AB.
   Apply biotin block - 15 minutes at room temperature.
   Wipe excess block.

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Cyclin D1) at 1:100 dilution and incubate for one hour at room temperature.
   Lot#_____ Exp Date_____

   For negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (Cyclin D1), and use this to make the 1:100 dilution. Apply to slides and incubate for one hour at room temperature.
   Lot#_____ Reconstituted Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody (Biotinylated horse anti-mouse) at a 1:500 dilution and incubate for 30 minutes at room temperature.
   Lot#_____ Reconstituted Date_____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label (Biogenex) antibody and incubate for 30 minutes at room temperature.
    Lot# _____ Exp. Date _____

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
    (Add 1 drop of DAB per ml of substrate)
    Lot#_____ Exp Date_____ New Kit yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 times	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

19. Coverslip

Updated 08/23/06